Original Article:
TNF-α Production Modulated by Aloe Vera Gel Extract and its Fractions in Candida Albicans Infected Macrophages

Zohre Farahnejad[1*], Davood Jamali[2,3]

1. Department of Medical Mycology, School of Medical Sciences, AJA University, Tehan, Iran.
2. Immunoregulation Research Center, Shahed University, Tehran, Iran.
3. Department of Immunology, Shahed University, Tehran, Iran.

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ABSTRACT

Background: Aloes have been used as medicinal plants for centuries. The immunomodulatory effect of Aloe vera has previously been shown. Meanwhile, TNF-α, as an inflammatory cytokine, plays an essential role in defense against invading pathogen. In the present study, the effects of A. vera extract gel and its fractions were investigated on the TNF-α production by macrophages against Candida albicans as an opportunistic microorganism in humans.

Materials and Methods: TNF-α was measured using Enzyme-Linked Immunosorbent Assay according to the instructions of the manufacturer (Biosource, Switzerland).

Results: The results showed that TNF-α level was increased by A. vera gel extract and some isolated fractions dose dependently. The A. vera gel extract in dilutions of 1:2, 1:5, 1:10 and 1:50 significantly increased the production of TNF-α. Likewise, R100 and R30 fractions of A. vera caused significant increase in TNF-α production. However, R10 and R5 fractions caused a reduction in TNF-α production as compared with that in control group.

Conclusion: These results showed that A. vera gel extracts and its fractions induce both immunostimulating and immunosuprressive effects on the production of TNF-α against Candida albicans. The efficacy of immunomodulatory of this herb is dose-dependent.

Introduction

Tumor Necrosis Factor-α (TNF-α) is a main mediator of the acute inflammatory which responses not only to gram-negative bacteria but also to other microbial infectious and is responsible for many of the systemic complications of severe infections. The major cellular source of TNF-α is activated mononuclear phagocytes. TNF-α stimulates endothelial cells and macrophage to secrete chemokines that enhance the affinity of leuko-

*** Corresponding Author:**
Zohre Farahnejad, PhD.
Address: *Department of Medical Mycology, School of Medical Sciences, AJA University, Tehan, Iran.*
Phone: *+98 (912) 2773149*
E-mail: *z.farahnejad@yahoo.com*

cyte integrins for their ligands and induce leukocyte chemotaxis and recruitment [1, 2].

Candida species are common causes of nosocomial infections, with an incidence that has significantly increased in the recent years. It has been reported that Candida is responsible for 9% of all nosocomial bloodstream infections and it is the most common cause of invasive mycoses [3-5]. Its crude mortality rate ranges from 40% to 60% and it is ranked as the fourth opportunist infectious agent after Staphylococcus aureus, Pseudomonas Spp, and Escherichia coli in hospitals [4, 6-8]. Despite the widespread use of antifungal drugs for prophylaxis, Candida Bloodstream Infection remains the most frequent life-threatening fungal disease. Several studies have reported different resistance mechanisms in Candida spp. and described relevant resistances; these studies have shown reduced susceptibility to antibiotics [9, 10]. There are different approaches to treatment and prevention of Candida albicans. The recent efforts have focused on immunotherapy and vaccines [11].

Also, Aloe vera (Family: Liliacea) is known as a medicinal herb and has shown a wide range of biological, pharmacological, and immunomodulatory activities. These activities may play an important role in the treatment of cancer and in prevention of life-threatening secondary infectious diseases occurring in immunosuppressive individuals [12, 13]. Also, many studies have demonstrated that Aloe can activate macrophages and its productions, i.e. cytokines, including IL-1, IL-2, IL6, IL-12, and TNF-α. Consequently, they stimulate antigen processing, non-specific immunity, wound healing, and resistance to infection and neoplasia [14, 15]. We previously reported that A. vera gel extract and some of its fractions induce cellular viability in Candida albicans infected macrophages [16]. In the present study, we made an attempt to show the effect of A. vera and its isolated fractions on the production of an important cytokine, i.e. TNF-α, against C. albicans in vitro model.

Materials and Methods

Microorganism and culture condition

Candada albicans Persian Type Culture Collection (PTCC, 50-27) isolated from our laboratory collection was used in the current study. C. albicans was cultured on Sabourads Dextrose Agar at 4°C [17]. The culture was repeated on sabouradto refresh Candida and the yield was shaked at 150 rpm. The cells were collected by centrifugation at 1200×g (5 centigrate degree) and washed three times with 10mM Phosphate Buffer Saline (PBS) 24 hours later.

Preparation of Aloe gel extract and fractions

Aloe gel obtained from the new leaf was mixed with distilled and deionized water with the ratio of 1:1 and centrifuged (14000 g/15min) to remove insoluble particles. Aloe gel extract in concentration of 1 g/mL was fractionated using Amicon Ultra-4 Centrifugal Filter Device (ACFD, Germany). Using this method, the extract was fractionated to 5 fractions based on their molecular weights; R100 (>100 kDa), R50 (100 kDa >R50 > 50 kDa), R30 (50 kDa >R30 > 30 kDa), R10 (30 kDa > R10>10 kDa), and R5 (10 kDa >R5 > 5 kDa). Aloe gel extract and its fractions were used in different dilutions according to the previously published methods [16].

Isolation of peritoneal macrophages

Inbred female Balb/c mice, 8-10 weeks age and 18-22 gr weight, were purchased from Razi Research Institute, Tehran, Iran. The mice were sacrificed using cervical dislocation and peritoneal exudates cells were harvested by lavage using cold PBS, as previously described [18]. Briefly, cells were washed twice, suspended in RPMI medium (Sigma, St. Louis, MO), and supplemented with 10% fetal calf serum (FCS). The suspensions were centrifuged at 1500 g and 4°C for 10 min. The supernatant was then discarded and RPMI with 10% FCS was added. The number of macrophages was adjusted to 4×105 MQ/ml using Neobar cytometer.

The 4×105 cells were added to each well of 96-well tissue culture microtiter plates (Falcon BD Labware, Franklin Lakes, and NJ) and incubated for 2h at 37°C under 5% CO2. After incubation, cultures were washed with warm normal saline (injectable grade at 37°C) to remove non-adherent cells. Then, macrophages were treated with 2×105 C. albicans per well and, subsequently, the RPMI with 10% FCS containing various doses of the extract and its fractions was added to the wells (five wells for each dose). The positive control cultures were five C. albicans-infected wells.

TNF-α measurement

TNF-α was measured using Enzyme-Linked Immunosorbent Assay according to the instructions of the manufacturer (Biosource, Switzerland). Supernatants of macrophage cell culture were collected after 12h incubation of the macrophages with various doses of extract and its fractions. A 96-well flat-bottomed microtiter plate was

pre-coated overnight with an anti-cytokine monoclonal antibody followed by blocking and several washings and then the standards and samples (collected supernatants from macrophage cultures) were added. After washings, the anti-mouse cytokine monoclonal antibody was added to each well and incubated for 2h. Substrate solution was then added followed by the addition of stop solution and the absorbance was read using a microtiterplate reader (ICNFlow, model: MK11) at wavelength at 450 nm.

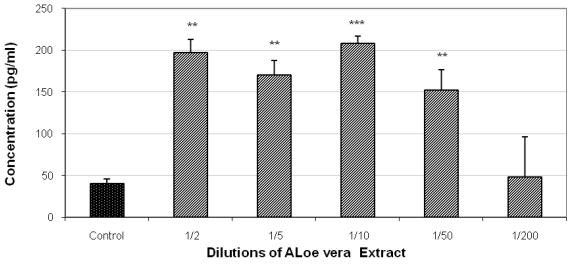
Statistical analysis of data

Analysis of Variance and student t-test were performed to determine significance levels among the different groups and controls.

Results

As shown in Figure 1, the production of TNF-α against C. albicans infection significantly increased in the presence of A. vera gel extract in various dilutions including 1:2, 1:5, 1:10, and 1:50 in comparison with that in uninfected controls (P=0.001, 0.002, 0.000, and 0.012, respectively). Also, Figure 2 shows that some dilutions of R100 fraction, including 1:2, 1:5, and 1:10, caused significant elevation in the production of TNF-α in comparison with those of the negative controls (p=0.006, 0.018, and 0.038, respectively), while no significant difference was observed in dilutions of 1:50 and 1:200 (p=0.133 and 0.260, respectively). Moreover, data presented in Figure 3 shows that using different dilutions of R50 fraction of A. vera gel extract did not alter TNF-α level.

Figure 4 shows that using 1:2 dilution of R30 fraction of A.vera gel extract significantly increased TNF-α production in comparison with that for negative control culture (p= 0.0151). Figure 4 also indicates that some dilutions of



IMMUNOREGULATION

Figure 1. The effect of different doses of A. vera gel extract on the production of TNF-α against C. albicans infection. *Denotes significant differences. *P<0.05, **P<0.01, and ***P<0.001 compared with Negative control: uninfected control group.

R30 fraction, including 1:10, caused significant decrease in TNF-α production (P=0.001). Also, as shown in Figure 5, all applied dilutions of R10 fraction, including 1:2, 1:5, and 1:50, caused significant reduction in the production of TNF-α in comparison with that for negative control culture (P=0. 006, 0.035, and 0.002, respectively). The production of TNF-α significantly decreased in applied dilutions of R5 fraction of A. vera including 1:2, 1:5, and 1:10 in comparison with that for negative control culture (P=0.004, 0.003 and 0.001, respectively) (Figure 6).

Discussions

C. albicans is one of the most frequent pathogens among the medically important Candida species, causing sever candidiasis in immunocompromised patients, i.e malignant, transplant, and ICU patients. There are different approaches to treatment and prevention of C. albicans. Recent efforts have focused on immunotherapy in C. albicans infection [11]. Previously, we showed an elevation in macrophage cell viability against C. albicans after A. vera treatment. The present study was car-

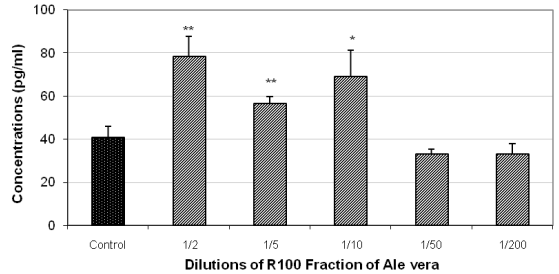


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Figure 2. The effect of different doses of R100 fraction of A. vera extract on the production of TNF-α against C. albicans infection.

*Denotes significant differences. *P<0.05, **P<0.01, and ***P<0.001 compared with Negative control: uninfected control group.

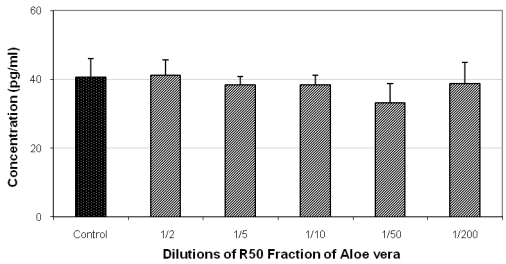

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Figure 3. The effect of different doses of R50 fraction of A. vera extract on the production of TNF-α against C. albicans infection.

*Denotes significant differences. *P<0.05, **P<0.01, and ***P<0.001 compared with Negative control: uninfected control group.

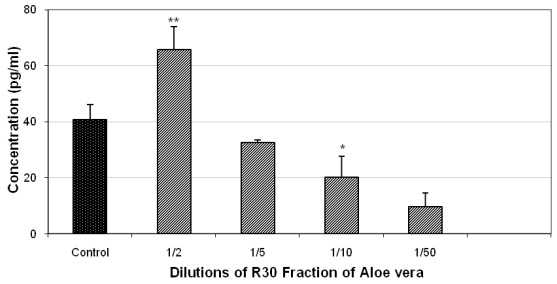

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Figure 4. The effect of different doses of R30 fraction of A. vera extract on the production of TNF-α against C. albicans infection.

*Denotes significant differences. *P<0.05, **P<0.01, and ***P<0.001 compared with Negative control: uninfected control group.

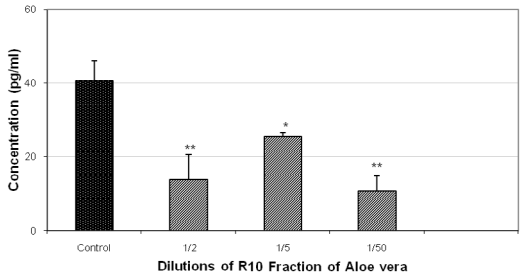

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Figure 5. The effect of different doses of R10 fraction of A. vera extract on the production of TNF-α against C. albicans infection.

*Denotes significant differences. *P<0.05, **P<0.01, and ***P<0.001 compared with Negative control: uninfected control group.

ried out to determine the effect of A. vera gel extract and its isolated fractions on TNF-α secretion by peritoneal macrophages against C. albicans.

The results indicated that A. vera gel extract induces a variety of both immunostimulating and immunosuprressive effects on the production of TNF-α by peritoneal macrophages. The optimum effects of A. vera gel extract on TNF-α secretion by peritoneal macrophages (about five folds of positive control group) were achieved in 1:2 to 1:10 dilutions. In addition, we performed fractionation of A. vera gel extract and isolated five fractions from A. vera based on molecular weight ranges. Among these fractions, R100 fraction showed stimulating effects on the production of TNF-α in 1:2 to 1:10 dilutions (about two folds of positive control group); R50 did not show any significant effect on TNF-α production whereas R30 showed mild effects so that in 1:2 dilution, it demonstrated stimulating effects and in 1:10 dilution, it showed suppressing effects.

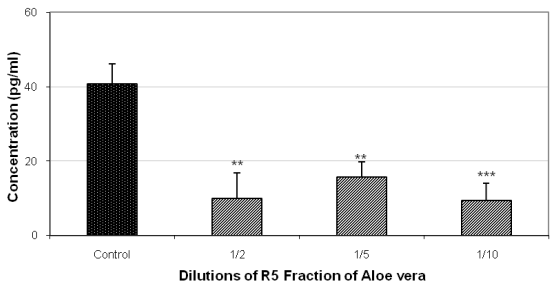

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Figure 6. The effect of different doses of R5 fraction of A. vera extract on the production of TNF-α against C. albicans infection.

*Denotes significant differences. *P<0.05, **P<0.01, and ***P<0.001 compared with Negative control: uninfected control group.

Our findings, which indicate that the high-molecular weight components of R100 fraction of A. vera gel extract induced more effect on TNF-α secretion by macrophages, are in agreement those of our last publication [16], in which R100 fraction revealed to induce more macrophage viability in comparison with other fractions, as well as the findings reported by Djeraba et al. [19]. They showed that acemannan, a beta-(1, 4)-acetylated mannan isolated from A. vera with 274-375 molecular weight, can increase macrophage activity [19]. This molecule was isolated in R100 fraction in our study. Findings of the present study and those of others suggest that the immunostimulatory effect of A. vera gel is related to high-molecular-weight components.

Our previous study also designated R50 as another fraction that contains components between 50 and 100 kDa molecular weights showing immunomodulatory activity by inducing macrophage cell viability [16], although in the present study, R50 did not show such an effect on TNF-α secretion by macrophages, but it should be noted that macrophage is a pluripotent effector cell and its activation is not limited to cytokine secretion.

Our results also demonstrated that other fractions containing lower molecular weights components, i.e R10 and R5, significantly decreased TNF-α secretion by peritoneal macrophages. This finding is consistent with our earlier findings that have shown the fractions containing lower molecular weights components did not show any alteration in the macrophage cell viability with all applied doses [16]. Recently, Das et al. isolated a 14 Kda protein from aloe leaf gel extract with antifungal and anti-inflammatory activity [12]. In our study, R10 fraction contained molecules between 10 and 30 kDa, including the 14 kDa isolated protein. It can be proposed that in ac-

cordance with Das et al study [12], R10 fraction shows anti-inflammatory effect. Overall, findings of the present study together with those of other studies suggest that the immunosuppressive effect of A. vera gel is related to low-molecular-weight components.

In conclusion, the current study proposed the principal immunomodulatory effect of A. vera gel extract and its fractions on TNF-α secretion by peritoneal macrophages against C. albicans. Our findings also indicated that R100 fraction, that contains high-molecular-weight components (MW>100 kDa), is the most immunostimulating and the low molecular weight components in R10 and R5 fractions are the most immunosuppressive and anti-inflammatory fractions of A. vera.

Due to the growing use of immunosuppressive drugs, long term broad spectrum antibiotic therapy, invasive medical devices, and complex surgical procedures, development of C.albicans infections is expected and to control this infection, access to appropriate methods of therapy, such as immunotherapy, is very important. Thus, A. vera is a candidate for this purpose. On the other hand, the low molecular weight components in A. vera gel are the good materials with anti-inflammatory properties and could be used for inflammation control.

Conflict of interest

The author reports no conflict of interest.

Acknowledgements

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